Clinica Chimica Acta, 166 (1987) 255–263
Elsevier

CCA 03826

Peroxisomal very long-chain fatty acid β-oxidation in human skin fibroblasts: activity in Zellweger syndrome and other peroxisomal disorders

R.J.A. Wanders [a], C.W.T. van Roermund [a], M.J.A. van Wijland [a], J. Heikoop [a], R.B.H. Schutgens [a], A.W. Schram [b], J.M. Tager [b], H. van den Bosch [c], B.T. Poll-Thé [d], J.M. Saudubray [d], H.W. Moser [e] and A.B. Moser [e]

[a] *Paediatric Department, University Hospital Amsterdam, Amsterdam (The Netherlands),*
[b] *Laboratory of Biochemistry, University of Amsterdam, Amsterdam (The Netherlands),*
[c] *Laboratory of Biochemistry, University of Utrecht, Utrecht (The Netherlands),*
[d] *Hôpital des Enfants Malades, Paris (France) and*
[e] *John F. Kennedy Institute, Baltimore, MD (USA)*

(Received 26 September 1986; revision received 21 February 1987; accepted after revision 22 February 1987)

Key words: Peroxisomal β-oxidation; Very long-chain fatty acid; Peroxisome; Zellweger syndrome; Adrenoleukodystrophy; Refsum disease

Summary

Since very long-chain fatty acids with a chain length of 24 carbons or more are known to accumulate in tissues and body fluids from patients with the cerebro-hepato-renal (Zellweger) syndrome, infantile Refsum disease, neonatal adrenoleukodystrophy and X-linked adrenoleukodystrophy, we studied very long-chain fatty acid oxidation in cultured skin fibroblasts from these patients. In this paper, we report that in accordance with earlier results the first step in the β-oxidation of the very long-chain fatty acid lignoceric acid (C24 : 0) primarily occurs in peroxisomes in control human skin fibroblasts. Furthermore, it was found that peroxisomal lignoceric acid β-oxidation was strongly deficient in fibroblasts from patients with Zellweger syndrome, infantile Refsum disease, neonatal and X-linked adrenoleukodystrophy, which explains for the accumulation of very long-chain fatty acids in all four disease entities. In Zellweger syndrome, infantile Refsum disease and neonatal adrenoleukodystrophy the impairment in peroxisomal very long-chain

Correspondence to: Dr R.J.A. Wanders, Department of Paediatrics, room FO-224, University Hospital Amsterdam, Academic Medical Centre, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.

0009-8981/87/$03.50 © 1987 Elsevier Science Publishers B.V. (Biomedical Division)

fatty acid β-oxidation is probably caused by a strong deficiency of all peroxisomal β-oxidation enzyme proteins due to a deficiency of peroxisomes.

Introduction

It is now clear that peroxisomes are involved in a number of metabolic processes including etherphospholipid biosynthesis, bile acid synthesis and fatty acid β-oxidation. The importance of peroxisomes in man is stressed by the existence of a group of diseases, the peroxisomal disorders, in which there is an impairment in one or more peroxisomal functions. The cerebro-hepato-renal (Zellweger) syndrome is generally considered to be the prototype of this newly recognized group of diseases. Other diseases belonging to this group are: the X-linked and neonatal forms of adrenoleukodystrophy, the classic and infantile forms of Refsum disease, hyperpipecolic acidaemia, pseudo-Zellweger syndrome, chondrodysplasia punctata (rhizomelic type), acatalasaemia and as recently discovered, hyperoxaluria type I (for reviews see refs. [1–4]).

Like mitochondrial fatty acid β-oxidation, peroxisomal fatty acid β-oxidation proceeds via successive steps of dehydrogenation, hydration, dehydrogenation and thiolytic cleavage. These reactions are catalyzed by the specific peroxisomal β-oxidation enzymes acyl-CoA oxidase (EC 1.3.99.3), the bifunctional protein with enoyl-CoA hydratase (EC 4.2.1.17) and 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) activities and 3-oxoacyl-CoA thiolase (EC 2.3.1.16). Rather than a duplicate of the mitochondrial β-oxidation system, peroxisomes have been implicated in the β-oxidation of a variety of metabolites including mono-unsaturated long chain fatty acids [5], very long-chain fatty acids [6], dicarboxylic acids [7], xenobiotics [8] and coprostanoic acids [9]. Since very long-chain fatty acids are known to accumulate in several of the peroxisomal disorders listed above, including Zellweger syndrome, infantile Refsum disease, X-linked and neonatal adrenoleukodystrophy (for review see ref. [3]), we have studied fatty acid β-oxidation in cultured skin fibroblasts, using palmitate (C16 : 0) and lignocerate (C24 : 0) as substrates. The results obtained are described in this paper.

Materials and methods

Cell culture conditions

Human skin fibroblasts were grown from skin biopsies taken from control individuals and patients in 25 cm^2, 75 cm^2 or 150 cm^2 culture flasks containing 5 ml, 15 ml or 30 ml HAM F-10 medium (Flow Laboratories, Irvine, Scotland), respectively, supplemented with 7.5% (v/v) fetal calf serum, 7.5% (v/v) newborn calf serum, 3 mmol/l glutamine, 26.2 mmol/l $NaHCO_3$, 100 U/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml fungizone in an atmosphere of 5% (v/v) carbon dioxide in air. At the time of cell harvest when the cells had reached the stage of confluency, the monolayer surface was washed twice with Hanks' Balanced Salt Solution (HBSS), followed by layering 1.0 ml/25 cm^2 0.25% (w/v) trypsin in

HBSS-buffer over the monolayer surface. When the cells detached, 5–10 ml HAM F-10 medium supplemented with fetal calf serum and newborn calf serum as described above was added and the content of the vial transferred to a plastic conical centrifuge tube. Subsequently, the cells were pelleted via centrifugation ($500 \times g_{av}$, 5 min). The pelleted cells were resuspended in Hanks' Balanced Salt Solution and centrifuged again, followed by two additional washings in phosphate-buffered saline (150 mmol/l NaCl, 10 mmol/l potassium phosphate, pH 7.4). All centrifugation steps were carried out at room temperature. The final cell pellet was taken up in a buffer containing 250 mmol/l sucrose plus 5 mmol/l morpholinopropane sulphonic acid (MOPS)-NaOH (final pH 7.4) and stored on ice until use. The cells showed a high degree of integrity as revealed by Tryphan-blue exclusion and the high degree of latency of the cytosolic enzyme lactate dehydrogenase ($\geq 90\%$).

Measurements of fatty acid oxidation in intact fibroblasts

In order to measure fatty acid oxidation in human skin fibroblasts, fibroblasts prepared as described above were incubated for 60 min at 37°C in a medium containing 250 mmol/l sucrose, 5 mmol/l MOPS-NaOH, pH 7.4, 2 mmol/l L-carnitine and 10 μmol/l palmitate or lignocerate added as α-cyclodextrin solution (final concentration of α-cyclodextrin 1 mmol/l). The latter substrates were prepared as 100 μmol/l stock solutions according to the following procedure: [1-^{14}C]palmitic acid or [1-^{14}C]lignoceric acid (50–60 mCi/mmol) dissolved in benzene or toluene were pipetted into glass tubes followed by evaporation to dryness under a stream of nitrogen at room temperature. Subsequently, 1 ml of a solution containing 100 mmol/l Tris–HCl plus 10 mmol/l α-cyclodextrin (final pH 8.5) was added. Full solubilization was obtained via a 30-min incubation in a sonicating water bath at room temperature. Reactions were started by adding a 20 μl portion of this solution to the incubation mixture (final volume 200 μl). In experiments where only peroxisomal β-oxidation activities were measured, carnitine was omitted from the medium and 5 mmol/l KCN added. Incubations were carried out in specially developed closed vessels in order to measure the production of $^{14}CO_2$ and water-soluble products. Reactions were terminated by addition of perchloric acid (final concentration: 500 mmol/l). Radioactive $^{14}CO_2$ was trapped in hyamine; water-soluble products were measured as described before [10].

In some experiments (Table I), the fatty acid was coated on the diatomateous earth, Celite as described by Singh et al [6]. In these experiments portions of the

TABLE I

Lignoceric acid β-oxidation in cultured skin fibroblasts from control individuals

Assay procedure as described in	Rate of lignoceric acid β-oxidation (pmol/min per mg protein)
Ref. [6]	2.9 ± 0.6 (4)
This paper	6.3 ± 2.8 (4)

Cultured skin fibroblasts were incubated in the standard reaction medium containing 2 mmol/l L-carnitine. Results as mean ± SD with the number of experiments in parentheses.

radiolabelled fatty acid in benzene or toluene were taken to dryness in glass vials under a stream of nitrogen at room temperature followed by the addition of Celite (100 mg/ml) in alcohol (final concentration in assay 10 mg/ml). After the vials were taken to dryness again, assay medium containing α-cyclodextrin was added and reactions were started by addition of fibroblast protein, essentially as described by Singh et al [6]. $^{14}CO_2$ and water-soluble products were measured as described above.

Very long-chain fatty acid measurements in cultured skin fibroblasts

Very long-chain fatty acids were determined as described before [11,12].

Materials

Nucleotides and enzymes were purchased from Boehringer (Mannheim, FRG) or Sigma (St. Louis, MO, USA). Radiochemicals were obtained from Amersham International (Amersham, UK) ([1-^{14}C]palmitic acid) and IRE, France ([1-^{14}C]lignoceric acid). Both compounds were found to be radiochemically pure as assessed by thin-layer chromatography. All other reagents were of analytical grade.

Patients

The patients studied in this report have been selected on the basis of established diagnoses based on clinical and biochemical grounds. The infantile Refsum patients studied represent case 2 and 3 from Scotto et al [13] (see also Poll-Thé et al [14]). The neonatal adrenoleukodystrophy patients have been described in detail by Kelley et al [15] and Wolff et al [16]. The Zellweger patients studied have been described elsewhere (Heymans et al [17]; Wanders et al [18]). The X-linked adrenoleukodystrophy patients studied showed the typical features described in literature (see refs. [19,20]). The classical Refsum patient is described in the following case history.

Case history

This female patient has an older brother and a younger sister also affected by Refsum disease. There is no consanguinity between the parents. The patient first noticed poor vision at night at 15 yr of age and retinitis pigmentosa was subsequently diagnosed. Her plasma phytanic acid level was 85.6 μg/ml (normal values: < 10) at that time, and the diagnosis Refsum disease was established. When her clinical status deteriorated at 22 yr of age, a diet low in phytanic acid was initiated. On evaluation at 27 yr of age she presented anosmia, bilateral constricted visual fields and pigmentary retinal degeneration. No hearing deficit was noted. Her gait was wide based with foot drop. A sensorimotor distal and symmetric neuropathy affecting the legs was present. Motor nerve conduction velocities were reduced in the lower extremities. During subsequent years she developed a moderate cerebellar ataxia. Plasma phytanic acid values ranged from 83–190 μg/ml. At 34 yr of age she is still able to work as a nurse.

Results

In Table I we studied the oxidation of lignoceric acid (*n*-tetracosanoic acid; C24 : 0) in cultured skin fibroblasts. Since lignoceric acid is practically insoluble in aqueous solutions, we adopted the procedure described by Singh and Kishimoto [21] to measure lignoceric acid β-oxidation. This method involves the use of α-cyclodextrin in the presence of Celite to solubilize the fatty acid. Rather than measuring the release of $^{14}CO_2$ from [1-^{14}C]lignoceric acid, we measured the production of $^{14}CO_2$ plus ^{14}C-labeled oxidation products as an index for lignoceric acid β-oxidation since it was found that $^{14}CO_2$ is only a minor product of fatty acid β-oxidation in cultured skin fibroblasts [22]. Using these experimental conditions lignoceric acid was found to be oxidized efficiently in intact human skin fibroblasts (Table I). Higher rates of lignoceric acid β-oxidation were found, however, when lignoceric acid was not first coated on Celite as in the original procedure [21] but instead added directly as fatty acid/α-cyclodextrin complex (see 'Discussion'). Based on these results the latter procedure was used in all subsequent experiments.

The results of Table II show that in the presence of L-carnitine palmitic acid is oxidized much faster than lignoceric acid. Omission of L-carnitine together with addition of 5 mmol/l KCN to block mitochondrial oxidative phosphorylation resulted in a strongly reduced rate of palmitate oxidation whereas lignoceric acid β-oxidation was affected only by 25%. These results suggest that in cultured skin fibroblasts lignoceric acid is primarily oxidized in peroxisomes, in accordance with the conclusion by Singh et al [6], whereas palmitate is primarily oxidized in mitochondria in human skin fibroblasts. As shown by the data in Table II, lignoceric acid β-oxidation was found to be strongly impaired in fibroblasts from Zellweger patients both under conditions of peroxisomal β-oxidation (KCN present) as well as under conditions of mitochondrial plus peroxisomal β-oxidation (carnitine present). Palmitate oxidation in the presence of carnitine was found to be unimpaired, however, in Zellweger fibroblasts (Table II).

TABLE II

Palmitate (C16 : 0) and lignocerate (C24 : 0) β-oxidation in cultured skin fibroblasts from controls and Zellweger patients

Substrate	Addition	Rate of fatty acid β-oxidation (pmol/min per mg protein) in fibroblasts from		
		Controls ($n = 4$)	Zellweger patients	
			1	2
Palmitate	Carnitine	82.1 ± 6.1	73.6	61.1
	KCN	4.2 ± 0.7	0.9	n.d.
Lignocerate	Carnitine	6.3 ± 2.8	0.4	0.8
	KCN	4.8 ± 0.9	0.1	0.2

For experimental details see 'Materials and Methods'. Results are expressed as mean ± SD with the number of different cell lines used within parentheses. n.d., not determined.

TABLE III

Peroxisomal lignoceric acid β-oxidation activity and the accumulation of very long-chain fatty acids in human skin fibroblasts from controls and patients with different peroxisomal disorders

Established diagnosis	C26/C22-fatty acids ratio	Peroxisomal lignoceric acid β-oxidation activity (pmol/min per mg protein)
Controls		
Mean ± SD		4.6 ± 0.92 (9)
Range	0.016–0.076 (23)	3.1–8.1
Zellweger syndrome		
1 Heymans et al [15], case 2	1.07	0.16
2 Heymans et al [15], case 9	0.88	0.11
3 Wanders et al [18], case 2	1.07	0.14
Neonatal adrenoleukodystrophy		
1 Wolff et al [14]	1.76	0.07
2 Kelley et al [13], case 3	1.60	0.12
3 Kelley et al [13], case 6	1.33	0.26
X-linked adrenoleukodystrophy		
1	0.58	0.29
2	0.35	0.24
Infantile Refsum disease		
1 Scotto et al [11], case 2	0.84	0.22
2 Scotto et al [11], case 3	0.49	0.52
Adult Refsum disease		
1	0.02	4.8

Lignoceric acid β-oxidation was measured in cultured skin fibroblasts as described in 'Materials and Methods' (5 mmol/l KCN present, no carnitine). Fibroblasts were only studied from patients with established diagnoses (see the original publications as indicated in the table for details on the patients). Each individual value in the table represents the mean of 2–4 separate experiments. Very long chain fatty acids were measured gaschromatographically as described in 'Materials and Methods'.

In Table III, we measured peroxisomal lignoceric acid β-oxidation activities (KCN present) not only in Zellweger fibroblasts but also in fibroblasts from patients with other peroxisomal disorders. The results of Table III indicate that the rate of peroxisomal lignoceric acid β-oxidation was not only impaired in fibroblasts from Zellweger patients but also in fibroblasts from patients with infantile Refsum disease, neonatal adrenoleukodystrophy and X-linked adrenoleukodystrophy (compare [6]). Normal rates of peroxisomal very long chain fatty acid oxidation were found in fibroblasts from a patient with the classic adult form of Refsum disease.

Discussion

Studies on the metabolism of very long-chain fatty acids such as lignoceric acid are hampered by the low solubility of these compounds in aqueous solutions. In order to circumvent these problems several procedures have been developed including the use of cyclodextrins [21]. Cyclodextrins are cylindrical polymers composed of 6–8 glucose moieties and are capable of solubilizing different water-insoluble

substances via complex formation [21]. In the original procedure described by Singh and Kishimoto [21] [1-^{14}C]lignoceric acid was coated on Celite, a diatomateous earth. A disadvantage of their experimental procedure is that each tube has to be prepared separately via a stepwise procedure involving addition of the radiolabelled fatty acid in chloroform or benzene, evaporation of the organic solvent, addition of Celite in ethanol and evaporation of the alcohol again. In our procedure, described in detail in 'Materials and Methods', coating on Celite is omitted. Instead, the fatty acid is directly complexed with α-cyclodextrin allowing reactions to be initiated by adding an aliquot of the fatty acid/α-cyclodextrin stock solution.

According to Singh et al [6] chain-shortening of very long-chain fatty acids such as lignoceric acid and cerotic acid occurs preferentially in peroxisomes. The finding that omission of carnitine together with addition of KCN led to a strong reduction in the rate of palmitate oxidation but not of lignocerate oxidation (Table II), supports the contention that in human skin fibroblasts the initiation of very long chain fatty acid β-oxidation, indeed, occurs primarily in peroxisomes. As shown in Table III, lignoceric acid β-oxidation was strongly impaired in fibroblasts from patients with Zellweger syndrome, X-linked adrenoleukodystrophy, neonatal adrenoleukodystrophy and infantile Refsum disease. Thus, the accumulation of very long chain fatty acids in serum and fibroblasts from these patients is caused by an impairment in the peroxisomal fatty acid β-oxidation system.

We have recently shown by immunoblotting [23,24] that there is a marked deficiency of the peroxisomal β-oxidation enzyme proteins acyl-CoA oxidase, bifunctional protein with enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities and 3-oxoacyl-CoA thiolase in liver from Zellweger and infantile Refsum patients. The results of subsequent continuous labelling and pulse/chase experiments [25] have shown that acyl-CoA oxidase is synthesized as a 72 kDa precursor that is converted to two polypeptides of M_r 52 and 20, whereas 3-oxoacyl-CoA thiolase is synthesized as a 44-kDa precursor that is converted to the 41 kDa mature protein. In fibroblasts from Zellweger and infantile Refsum patients, the precursors of the two enzymes are formed normally but in the absence of peroxisomes no processing to the mature forms occurs [25]. Instead, the newly synthesized precursors are rapidly degraded, in accordance with the deficiency of cross-reactive material in immunoblots of liver from the patients [23,24]. These findings thus provide an explanation for the apparent deficiency in peroxisomal β-oxidation as found in Table III.

Recent evidence suggests that peroxisomes are not only deficient in liver from patients with Zellweger syndrome [26] and infantile Refsum disease [27,28], but also in liver from patients with neonatal adrenoleukodystrophy [15,29]. Furthermore, as in fibroblasts from Zellweger and infantile Refsum patients [24], particle-bound catalase is also strongly deficient in fibroblasts from patients with neonatal adrenoleukodystrophy [30], suggesting that as in Zellweger syndrome and infantile Refsum disease the accumulation of very long chain fatty acids in neonatal adrenoleukodystrophy results from an impairment in peroxisomal fatty acid β-oxidation due to a deficiency of all peroxisomal β-oxidation enzyme proteins.

Available evidence suggests that in X-linked adrenoleukodystrophy the defect in

peroxisomal functioning is restricted to the oxidation of very long-chain fatty acids [6]. Indeed, peroxisomes have been found to be abundant in liver from the patients and there is no accumulation of abnormal bile acids, pipecolic acid and/or phytanic acid [6]. Recent evidence by Hashmi et al [31] suggests that the primary defect in X-linked adrenoleukodystrophy is at the level of lignoceroyl-CoA ligase, the enzyme responsible for the activation of lignoceric acid to lignoceroyl-CoA (see also refs. [4,32]).

Acknowledgements

This study was supported by grants from the Netherlands Organization for Pure Scientific Research (ZWO) under auspices of the Netherlands Foundation for Fundamental Medical Research (Medigon) and The Princess Beatrix Fund (The Hague, The Netherlands). Dr. J.A.J.M. Bakkeren is gratefully acknowledged for C26/C22-measurements. The authors wish to thank Paul Bentlage, Ellen Meyboom and Annie Van den Put for expert technical assistance and Anja Aaldering and Truus Klebach for typing the manuscript.

References

1 Kelley RI. The cerebro-hepato-renal syndrome of Zellweger, morphologic and metabolic aspects. Am J Med Gen 1983;16:503–517.
2 Goldfischer S, Reddy JK. Peroxisomes (microbodies) in cell pathology. Int Rev Exp Pathol 1984;26:45–84.
3 Schutgens RBH, Heymans HSA, Wanders RJA, Van den Bosch H, Tager JM. Peroxisomal disorders: a newly recognized group of genetic diseases. Eur J Pediatr 1986;144:430–440.
4 Wanders RJA, Schutgens RBH, Heymans HSA, et al. Biochemical analysis in peroxisomal disorders. In: Fahimi HD, Sies H, eds. Peroxisomes in biology and medicine. Berlin: Springer Verlag, 1987; pp 341–352.
5 Bremer J, Osmundsen H. Fatty acid oxidation and its regulation. In: Numa S, ed. Fatty acid metabolism and its regulation. Amsterdam: Elsevier Science Publishers,1984;113–154.
6 Singh I, Moser AE, Goldfischer S, Moser HW. Lignoceric acid is oxidized in the peroxisome: implications for the Zellweger syndrome and adrenoleukodystrophy. Proc Natl Acad Sci USA 1984;81:4203-4207.
7 Mortensen PB, Gregersen N, Rasmussen K. The β-oxidation of dicarboxylic acids in isolated mitochondria and peroxisomes. J Inher Metab Dis 1983;6 (Suppl 2):123–124.
8 Yamada J, Horie S, Watanabe T, Suga T. Participation of peroxisomal β-oxidation system in the chain-shortening of a xenobiotic acyl compound. Biochem Biophys Res Commun 1984;125:123–128.
9 Kase F, Björkhem I, Pedersen JI. Formation of cholic acid from 3α,7α,12α-trihydroxy-5β-coprostanoic acid by rat liver peroxisomes. J Lipid Res 1983;24:1560–1567.
10 Wanders RJA, Van Roermund CWT, De Vries CI, et al. Peroxisomal β-oxidation of palmitoyl-CoA in human liver homogenates and its deficiency in the cerebro-hepato-renal (Zellweger) syndrome. Clin Chim Acta 1986;159:1–10.
11 Bakkeren JAJM, Monnens LAH, Trijbels JMF, Maas JM. Serum very long chain fatty acid pattern in Zellweger syndrome. Clin Chim Acta 1984;138:325–331.
12 Govaerts L, Bakkeren JAJM, Monnens LAH, Maas JM, Trijbels JMF. Disturbed long chain (C24-C26) fatty acid pattern in fibroblasts of patients with Zellweger syndrome. J Inher Metab Dis 1985;8:5–8.
13 Scotto JM, Hadchouel M, Odièvre M, et al. Infantile phytanic acid storage disease, a possible variant of Refsum's disease: three cases, including ultrastructural studies of the liver. J Inher Metab Dis 1982;5:83–90.

14 Poll-Thé BT, Saudubray JM, Ogier H, et al. Infantile Refsum's disease: biochemical findings suggesting multiple peroxisomal dysfunction. J Inher Metab Dis 1986;9:169–174.
15 Kelley RI, Datta NS, Dobyns WB, et al. Neonatal adrenoleukodystrophy: new cases, biochemical studies and differentiation from Zellweger and related polydystrophy syndromes. Am J Med Gen 1986;23:869–901.
16 Wolff J, Nyhan WL, Powell H, et al. Myopathy in an infant with a fatal peroxisomal disorder. Pediatr Neurol 1986;2:141–146.
17 Heymans HSA, van den Bosch H, Schutgens RBH, et al. Deficiency of plasmalogens in the carebro-hepato-renal (Zellweger) syndrome. Eur J Pediatr 1984;142:10–15.
18 Wanders RJA, Smit W, Heymans HSA, et al. Age-related accumulation of phytanic acid in plasma from patients with the cerebro-hepato-renal (Zellweger) syndrome. Clin Chim Acta;in press.
19 Moser HW, Moser AB, Singh I, O'Neill BP. Adrenoleukodystrophy: survey of 303 cases: biochemistry, diagnosis, and therapy. Ann Neurol 1984;16:628–641.
20 Powers J. Adrenoleukodystrophy (adreno-testiculo-leuko-myeloneuropathic complex). Clin Neuropathol 1985;4:181–199.
21 Singh I, Kishimoto Y. Effect of cyclodextrins on the solubilization of lignoceric acid, ceramide, and cerebroside, and on the enzymatic reactions involving these compounds. J Lipid Res 1983;24:662–665.
22 Veerkamp JH, van Moerkerk HTB, Glatz JCG, et al. [$^{14}CO_2$]Production is no adequate measure of ^{14}C fatty acid oxidation. Biochem Med Metab Biol 1986;35:248–259.
23 Tager JM, Ten Harmsen van der Beek WA, Wanders RJA, et al. Peroxisomal β-oxidation enzyme proteins in the Zellweger syndrome. Biochem Biophys Res Commun 1985;126:1269–1275.
24 Wanders RJA, Schutgens RBH, Schrakamp G, et al. Infantile Refsum's disease: absence of catalase-containing particles (peroxisomes), alkyldihydroxyacetone phosphate synthase and peroxisomal β-oxidation enzyme proteins. Eur J Pediatr 1986;145:172–175.
25 Schram AW, Strijland A, Hashimoto T, et al. Biosynthesis and maturation of peroxisomal β-oxidation enzymes in fibroblasts in relation to the Zellweger syndrome and infantile Refsum disease. Proc Natl Acad Sci USA 1986;83:6156–6158.
26 Goldfischer S, Moore CL, Johnson AB, et al. Peroxisomal and mitochondrial defects in the cerebro-hepato-renal syndrome. Science 1973;182:62–64.
27 Ogier H, Roels F, Cornelis BT, et al. Absence of hepatic peroxisomes in a case of infantile Refsum's disease. Scand J Clin Lab Invest 1985;45:767–768.
28 Roels F, Cornelis A, Poll-Thé BT, et al. Hepatic peroxisomes are deficient in infantile Refsum disease. A cytochemical study of 4 cases. Am J Med Genet 1986;25:257–271.
29 Goldfischer S, Collins J, Rapin I, et al. Peroxisomal defects in neonatal onset and X-linked adrenoleukodystrophy. Science 1985;227:67–70.
30 Wanders RJA, Schutgens RBH, Schrakamp G, et al. Neonatal adrenoleukodystrophy: impaired plasmalogen biosynthesis and peroxisomal β-oxidation due to a deficiency of catalase-containing particles (peroxisomes) in cultured skin fibroblasts. J Neurol Sci 1987;77:331–340.
31 Hashmi M, Stanley W, Singh I. Lignoceroyl-CoASH ligase: enzyme defect in fatty acid β-oxidation system in X-linked childhood adrenoleukodystrophy. FEBS Lett 1986;196:247–250.
32 Wanders RJA, van Roermund CWT, van Wijland MJA, et al. Peroxisomal fatty acid β-oxidation in human skin fibroblasts: X-linked adrenoleukodystrophy, a peroxisomal very long chain fatty acyl-CoA synthetase deficiency. J Inher Metab Dis 1987; in press.